bio-protocol Preprint

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Sphere culture

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An abbreviated version of this protocol was published in Science Signaling in Oct 2020

SMAR1 repression by pluripotency factors and consequent chemoresistance in breast cancer stem-like cells is reversed by aspirin

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Detailed protocol

For preparing cancer stem cell-enriched spheres from cancer cells, breast cancer cell lines of low passage number were utilized. These cells were routinely observed for optimum health prior to seeding. For sphere culture, single cells were plated in 6-well ultralow attachment plates at a density of $2.5x10^4$ viable cells per well. The cells were cultured for 7 days in Dulbecco's modified Eagle's medium /F12 (DMEM/F12) (without serum) with 5 µg/mL of bovine insulin, 20 ng/mL of recombinant epidermal growth factor (EGF), 20 ng/mL of basic fibroblast growth factor (bFGF), 1X B27 supplement and 0.4% bovine serum albumin (BSA) to obtain the primary spheres. After 7 days, the primary spheres were collected by gentle centrifugation (800 rpm), and enzymatically dissociated using 1X Trypsin-EDTA solution to obtain single cells. These single cells were re-plated in the above-mentioned serum-free medium at a density of $2.5x10^4$ viable cells per well and cultured for additional 7-8 days to obtain the secondary spheres. Sphere-forming efficiency was determined by counting number of spheres under a light microscope at 10X magnification.

Steps:

1. Prepare the DMEM/F12 serum-free media using Dulbecco's modified Eagle's medium /F12 (DMEM/F12) with 5 µg/mL of bovine insulin, 20 ng/mL of recombinant epidermal growth factor (EGF), 20 ng/mL of basic fibroblast growth factor (bFGF), 1X B27 supplement and 0.4% bovine serum albumin (BSA). Do not add serum.
2. Take breast cancer cell lines of low passage number, routinely observe the cells for optimum health prior to seeding as spheres.
3. Make single cell suspension of the cells, count the cells and measure cell viability.
4. Take $2.5x10^4$ viable cells, dissolve in 1 ml DMEM/F12 serum-free media and add to each well of the 6-well ultralow attachment plates. Add another 1 ml DMEM/F12 serum-free media to each well. Each well of the 6-well ultralow attachment plates will have 2 ml of final media volume and a density of $2.5x10^4$ viable cells.
5. Culture the cells in 37°C in a humidified incubator containing 5% CO_2 for 7 days to obtain the primary spheres.
6. Routinely observe the cells for formation of spheres.
7. 1 ml DMEM/F12 serum-free media can be added to each well if needed after 2-3 days. Please add the media gently to not disturb the growing spheres.
8. After 7 days, collect the primary spheres by gentle centrifugation (800 rpm) and enzymatically dissociate the spheres using 1X Trypsin-EDTA solution to obtain single cells. Do not keep the cells in Trypsin-EDTA solution for more than 3-4 minutes.
9. Wash the cells with 1X PBS, count the cells and measure cell viability.
10. Re-seed $2.5x10^4$ viable cells in each well of 6-well ultralow attachment plates in DMEM/F12 serum-free media with a final volume of 2 ml of media in each well.
11. Culture the cells in 37°C in a humidified incubator containing 5% CO_2 for another 7-8 days to obtain the secondary spheres.
12. Routinely observe the cells for formation of spheres and optimum health. 1-2 ml of DMEM/F12 serum-free media can be added to each well if needed. Please add the media gently to avoid disturbing the growing spheres.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Das, T. (2020). Sphere culture. Bio-protocol Preprint. bio-protocol.org/prep674.
2. Bhattacharya, A., Mukherjee, S., Khan, P., Banerjee, S., Dutta, A., Banerjee, N., Sengupta, D., Basak, U., Chakraborty, S., Dutta, A., Chattopadhyay, S., Jana, K., Sarkar, D. K., Chatterjee, S. and Das, T.(2020). SMAR1 repression by pluripotency factors and consequent chemoresistance in breast cancer stem-like cells is reversed by aspirin . Science Signaling 13(654). DOI: 10.1126/scisignal.aay6077